SPECIFIC CHANGES IN THE PANCREATIC EXPRESSION OF THE INTERLEUKIN 1 FAMILY OF GENES DURING EXPERIMENTAL ACUTE PANCREATITIS

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Interleukin 1β (IL-1β) is produced in large amounts during acute pancreatitis and is believed to play a primary role in determining pancreatitis severity and the degree of pancreatic tissue destruction. This study was undertaken to characterize intrapancreatic production of IL-1β and the remainder of the IL-1 family of genes during sterile acute pancreatitis. Moderate or severe necrotizing pancreatitis was induced by the intraperitoneal injection of a cholecystokinin analogue or the feeding of a choline deficient diet, respectively. Animals were killed during the progression of pancreatitis with severity scored by histological grading and serum amylase concentration. The expression of IL-1β, IL-1 Receptor 1 (IL-1R1), IL-1R2, IL-1R antagonist (IL-1Ra), and ICE mRNA within the pancreas was examined by quantitative differential RT-PCR. Corresponding intrapancreatic and serum proteins were measured by enzyme-linked immunosorbent assay (ELISA). There was constitutive expression of pancreatic IL-1R1, IL-1R2, IL-1Ra, and ICE but not IL-1β. As pancreatitis developed, mRNA for IL-1β, IL-1Ra, and ICE increased in parallel with the degree of pancreatitis severity (all $P < 0.001$ vs baseline) while mRNA for both receptors remained stable (P = NS). Intrapancreatic and systemic IL-1β and IL-1Ra protein also increased as pancreatitis developed (both $P < 0.001$) with tissue levels being continuously greater than serum. This study demonstrates that sterile, endotoxin-free acute pancreatitis induces the upregulation of specific members of the IL-1 family of genes including production of large amounts of IL-1β and its receptor antagonist within the pancreatic parenchyma. These changes are indicative of pancreatitis severity are not model dependent.

Acute pancreatitis is a clinical entity which has a non-infectious inflammatory reaction associated with autodigestion of the pancreas as its central pathophysiological cause.[1-4] Several recent studies have implicated the pro-inflammatory cytokine IL-1β in the development of pancreatic destruction during moderate and severe acute pancreatitis. When the activity of this multifunctional cytokine is blocked with recombinant IL-1 receptor antagonist (IL-1Ra), pancreatitis severity and inflammation is attenuated, acinar cell necrosis is lessened, and mortality is dramatically reduced.[5-7] Subsequent studies in transgenic mice possessing a homozygous deletion of the type 1 IL-1 receptor (IL-R1) have demonstrated that maximal pancreatic destruction could not be produced during pancreatitis without this active receptor.[8] Furthermore, the lethality of severe necrotizing pancreatitis was reduced by 70% in animals in which the gene encoding for IL-1 converting enzyme (ICE) had been knocked out.[9] Although IL-1 activity is not necessary for the initiation of pancreatitis, there is sufficient data to implicate this cytokine and its family of genes as a promoter of the detrimental processes ongoing within the pancreatic parenchyma.

IL-1β is unique in that its production is dependent upon cleavage of intracellular pro-IL-1 to its active form by ICE, a cysteine protease.[10,11] Additionally, IL-1 is the only cytokine to be associated with its own naturally occurring receptor antagonist (IL-1Ra). The production of these two proteins is known to be regulated by many of the same factors (i.e. endotoxin) which are responsible for inducing the production of IL-1β.[10,12] Less is known, however, about their production during non-infectious disease processes such as acute pancreatitis. Although strong evidence exists that IL-1β plays an important role in the pathogenesis of acute pancreatitis, its production in this digestive organ has not been examined in detail. This study was undertaken to examine specific changes in one or more of members of the IL-1 family of genes

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within the pancreatic parenchyma during the development of sterile acute pancreatitis.

RESULTS

Development of pancreatitis

Acute edematous pancreatitis was demonstrable 1 h following the initial caerulein injection and progressively worsened over 6 h to include partial necrosis as determined by histological scoring and elevations in serum amylase (both $P < 0.01$ vs baseline, Fig 1A). Haemorrhagic necrotizing pancreatitis developed over a period of 2 days in the CDE diet fed animals, with significant elevations in amylase and histologic scores first detectable at the 24 hour time point (both $P < 0.01$ vs baseline) reaching severe necrosis and inflammation at 72 hours (Fig. 1B).

Baseline intrapancreatic mRNA

All animals demonstrated constitutive mRNA for IL-1R1 and IL-1R2 within the pancreatic parenchyma.

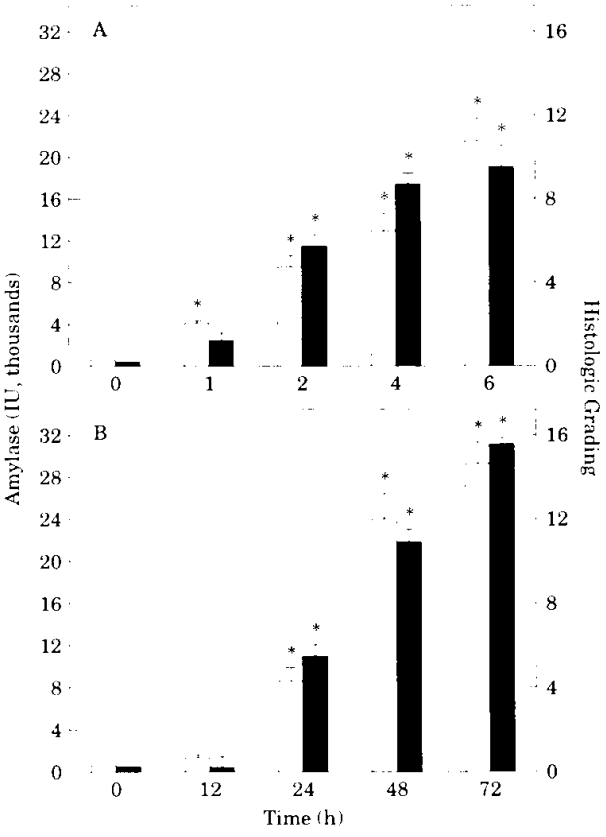

Figure 1. Assessment of pancreatitis severity.

Serum amylase (□) and blind histological grading (■) are shown during the development of moderate (A, caerulein-induced) and severe (B, CDE diet-induced) necrotizing pancreatitis. *$P < 0.01$ vs baseline

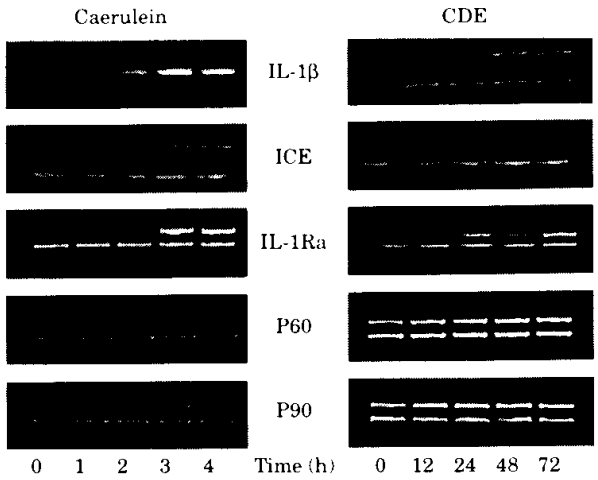

Figure 2. IL-1 gene family expression during moderate (caerulein-induced) and severe (CDE diet-induced) necrotizing pancreatitis.

The cDNA products are shown during the progression from baseline to severe pancreatitis. The internal standard β-actin was co-amplified with each gene examined.

Messenger RNA for ICE, and IL-1Ra was also present but at relatively lower concentrations (Fig. 2). There was no constitutive IL-1β mRNA detectable. These findings were independent of animal age, sex, or strain.

Changes in pancreatic mRNA during pancreatitis

During the development and progression of acute pancreatitis, mRNA for IL-1β and IL-1Ra was rapidly upregulated, peaking coincident with pancreatitis severity (both $P < 0.001$ vs baseline, Figs 2 and 3). ICE message was also increased although to a lesser degree. Contrary to these findings, intrapancreatic message for both receptors remained constant despite the development of pancreatitis (P = NS).

Intrapancreatic and serum protein levels by ELISA

Intrapancreatic and serum concentrations of IL-1β and IL-1Ra were at or below the sensitivity of our ELISA (12 pg ml) in all animals at baseline. Elevation of both proteins within pancreatic tissue was noted early in the development of pancreatitis which continued to increase as pancreatitis severity worsened (both $P < 0.001$ vs baseline, Fig. 4). Serum levels for IL-1β and IL-1Ra showed a similar rise as pancreatitis developed, however, pancreatic concentrations remained significantly higher than serum at each time point (both $P < 0.001$ vs baseline and $P < 0.05$ vs intrapancreatic). Although the more severe CDE model of pancreatitis typically induced higher levels of IL-1β and IL-1Ra, the production of these cytokines was not model dependent.

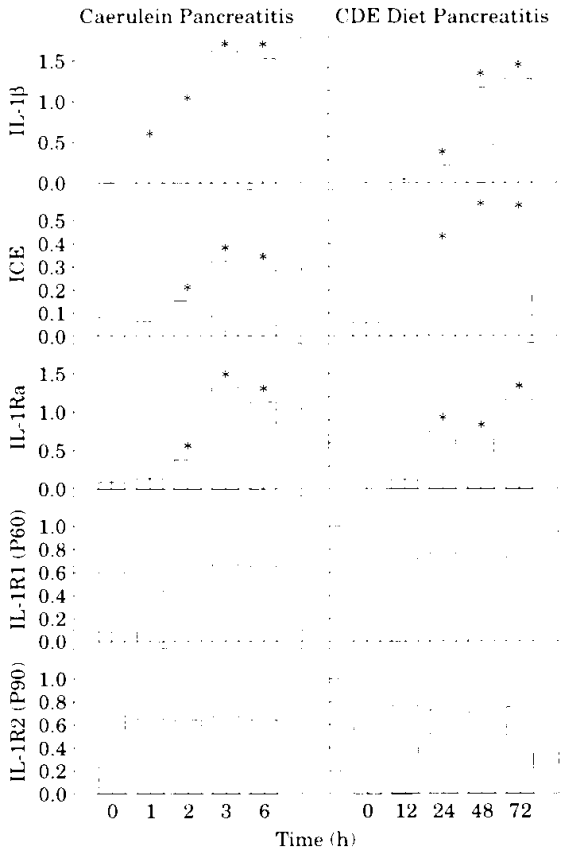

Figure 3. Quantification of intrapancreatic mRNA during moderate (rapid developing) and severe (slow develioping) necrotizing pancreatitis.

The cDNA products for each member of the IL-1 gene family were determined by computerized optical scanning and the ratio of targets to internal control was calculated. There was a significant increase in the amount of IL-1β, IL-1Ra, and ICE as pancreatitis developed ($^*P < 0.001$ vs baseline). Both IL-1R1 and IL-1R2 remained at baseline levels. The observed changes were not model dependent.

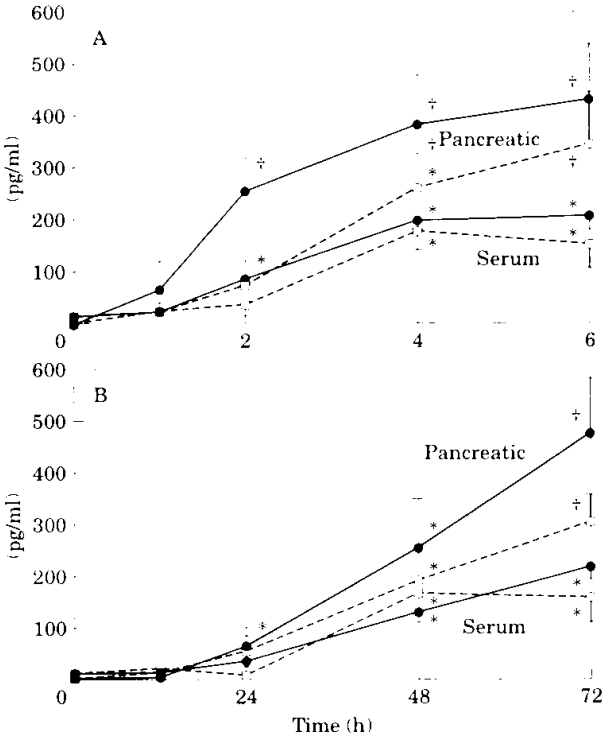

Figure 4. Intrapancreatic and serum IL-1β (- - □ - -) and IL-1Ra (—●—) by ELISA during moderate (A) and severe (B) pancreatitis.

The concentration of IL-1β and IL-1Ra within pancreatic tissue rises faster and obtains maximal values approximately three times higher than corresponding serum levels ($^*P < 0.001$ vs baseline, $^\dagger P < 0.05$ vs serum). The rise in both of these proteins mimics the increase in pancreatitis severity. As seen during endotoxin challenge, the concentration of IL-1Ra typically exceeds that of IL-1β.

DISCUSSION

Messenger RNA for IL-1β and its associated protein are not detectable in the normal pancreas. Once the cascade of acinar cell destruction and enzyme release has begun, the message for this cytokine quickly becomes apparent with the amount increasing as pancreatitis worsens. The progressive rise in serum IL-1β protein concentrations in these models of acute pancreatitis has been shown previously[13,16] and is typical of pro-inflammatory cytokine production in severe clinical pancreatitis.[17–20] The concentration of IL-1β within the pancreatic parenchyma, however, has not been established. This study allows time-matched comparisons between concentrations of IL-1β within the serum and pancreatic tissue providing for two important observations. First, the rate of rise within the tissue is significantly greater than it is within the serum. Second, the maximal concentration of IL-1β within the tissue is approximately three-fold higher than in the systemic circulation. Both observations are consistent with production within the pancreas, while the high levels seen within the tissue may give some insight as to why blockade of this cytokine has such a protective effect on pancreatic tissues during severe pancreatitis.

The signalling mechanism responsible for the production of IL-1β during the progression of acute pancreatitis also has direct effects on the induction of ICE and IL-1Ra genes. In fact, serum and tissue concentrations of IL-1Ra typically exceeded those of the agonist IL-1β. These findings are consistent with previous in vitro studies demonstrating that the production of IL-1β by endotoxin-stimulated macrophages is accompanied by an increase in the activity of ICE and a concomitant production of IL-1Ra.[10,12] Importantly, the models of pancreatitis utilized in the present study are known to be endotoxin and

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bacteria free.[21] Moreover, each pancreas in this study demonstrated no growth on routine culture. The actual signal for this process remains unclear but has been suggested to be secondary to activated enzymes.[16,21] Recent studies in our laboratory, however, have failed to demonstrate the ability of activated pancreatic enzymes to induce the production of cytokines from quiescent macrophages in vitro (unpublished observations).

The changes in the IL-1 family of genes appears to be specific rather than generalized. Although mRNA for both the type one and type two IL-1 receptors is expressed in moderate amounts within normal pancreata, the severe inflammation, necrosis, and edema of acute pancreatitis has no effect on the expression of these genes despite dramatic changes in other gene family members. This may be a reflection of the cellular make up of the pancreas which consists of more than 90% acinar cells which express these receptors. The production of IL-1 (and probably ICE), however, is most likely not originating from these cells, but from leukocytes which increase in numbers rapidly during the progression of pancreatitis. We have previously used immunohistochemistry to demonstrate that the vast majority of TNF-α produced within the pancreas originated within neutrophils and macrophages which infiltrate the gland.[16] Staining for IL-1β in a similar fashion shows the absence of staining within normal pancreata and a large infiltration of leukocytes which stain for the protein as pancreatitis worsens (data not shown).

The importance of the pro-inflammatory cytokines in the pathogenesis of acute pancreatitis is becoming increasingly apparent. We have demonstrated that IL-1β is produced within the pancreas in a predictable manner which is not model dependent. Although the link between IL-1β production and the development of pancreatic vacuolization, oedema, inflammation, and eventual acinar necrosis and apoptosis remains to be delineated, accumulating evidence suggests a major role for this cytokine family in the development and progression of acute pancreatitis.

MATERIALS AND METHODS

Animal model

Animal studies were performed at an AAALAC accredited facility in accordance with the Department of Laboratory Animal Medicine at the University of South Florida. Acute pancreatitis was induced by one of two well established models previously shown to be endotoxin free.[5,6,21] A rapidly progressive acute edematous pancreatitis was employed in 60 adult male NIH Swiss mice (32 ± 0.3 g) by the intraperitoneal (IP) injection of caerulein (50 μg kg h × 4), a cholecystokinin analogue.[5,22] Twelve animals were killed at 0 (baseline control), 1, 2, 4, and 6 h by exsanguination via cardiocentesis following pentobarbital anaesthesia (50 mg/kg i.p.).

A slowly progressive, severe, haemorrhagic, necrotizing pancreatitis was induced in 60 young female mice (15.4 ± 0.6 g) by the feeding of a choline-deficient, ethionine-supplemented (CDE) diet (Harlan Teklad, Madison WI) for 72 h.[6] Animals were fasted overnight prior to beginning the diet and were allowed water *ad libitum*. Feeding trays were changed every 6 h to assure sanitary conditions. Animals were killed at 0, 12, 24, 48, and 72 h as above.

All pancreata were immediately excised and divided for light microscopy and isolation of protein and total RNA. The severity of pancreatitis was established by blinded light microscopic histologic grading of haematoxylin and eosin (H&E) stained sections by a single investigator as previously described.[5,6,23] Serum amylase was determined on an automated Kodak Ectachem 700 analyser (Kodak, Rochester, NY).

Cellular RNA extraction

Total RNA was immediately isolated from all pancreata by guanidium thiocyanate acid phenol extraction as previously described.[16,24] The RNA preparations were controlled by minigel agarose electrophoresis with visualization of the 18S and 28S ribosomal RNA bands after ethidium bromide staining.

Measurement of cytokine gene expression by quantitative RT-PCR

Total RNA (2 μg) from each sample was reversed transcribed according to the SuperScript II Preamplification System (Gibco, Gaithersburg, MD) protocol using oligo $(dt)_{15}$ to prime the reverse transcriptase. The prepared cDNA was subjected to PCR with murine-specific primers for IL-1β,

TABLE 1. Murine cytokine cDNA primer sequences

Cytokine	Sense primer (5′ → 3′)	Anti-sense primer (5′ → 3′)	Product size
IL-1β	CAGGATGAGATGAGCACC	CTCTGCAGACTCAAACTCCAC	447
IL-1Ra	CTTCTGGGAAAAGACCCTGCA	ACAGGCAGCTGACTCAAAGCT	473
ICE	GATTCTAAAGGAGGACATCC	GTACATAAGAATGAACTGGA	930
IL-1R1	GAGGATAAAGGGGAGAGCAGTG	ATTCAAAAGGCAGGAGAGACAA	377
IL-1R2	TTTCTGCTTTCACCACTCCA	TTTCAGGTCAGGGCACACTA	473
β-Actin	GTGGGCCGCTCTAGGCACCA	CGGTTGGCCTTAGGGTTCAGGGGGG	245

IL-1β and IL-1Ra were purchased from Stratagene (La Jolla, CA) and Ransom Hill Bioscience (Ramona, CA) respectively. IL-1R1 and IL-1R2 were provided by Thomas Kline, Ph.D. at the University of South Florida, Tampa. ICE was a gift from Vertex Pharmaceuticals (Cambridge, MA).

ICE, IL-1Ra, IL-1RI, IL-1RII, and β-actin (Table 1). All RT products were co-amplified for 30 cycles in the presence of 5′ and 3′ primers for both the mRNA in question and β-actin in a UNO-Thermoblock (Biometra, Tampa, FL). The reaction products were subsequently visualized by electrophoresis in 2.5% Metaphor agarose (FMC Bioproducts, Rockland, ME) containing ethidium bromide. Ultraviolet illumination was used to visualize the DNA bands and the gels were photographed digitally and stored on computer disc. Band intensity was determined by optical density with specific message/β-actin cDNA ratios compared using Sigma Scan and Sigma Plot software (Jandel Scientific, San Rafael, CA). All primers are known to span at least one intron. The internal standard (β-actin) has previously been shown by our laboratory to be linear throughout the time course of both experimental models used while maintaining a linear relationship with the individual mRNA species in question from 20 through 45 amplification cycles.[16] Restriction digest of the IL-1β, ICE, IL-1Ra, IL-1RI, IL-1RII products with *Pst*I, *Hind*III, *Ava*II, *Pvu*II, and *Alu*I, respectively, yielded the expected base pair fragments (data not shown).

IL-1β and IL-1Ra protein quantification via ELISA

Serum and intrapancreatic concentrations of IL-1β and IL-1Ra were determined using standard ELISA methodology[5,6] using commercially available kits purchased from GEnzyme (Cambridge, MA). Pancreatic homogenate protein concentration was standardized to 7.5 mg/dl to allow comparison to serum (7.5 mg/dl). All samples were run in triplicate with optical density determined at 405 nm using an automated plate reader and analyzed using an Immunosoft software package (Dynatech Laboratories, Chantilly, VA).

Analysis of data

Results are expressed as means ± standard error of the mean. All data analysis was performed using the EPISTAT statistical program (Epistat Services, Richardson, TX) applying the Student's *t*-test.

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